An Engineered Rare Codon Device for Optimization of Metabolic Pathways

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Table S1. Primers used in this study

Primer name	sequence (5' to 3')
2luc-NcoI-F	**CCCCCCATGG**CGAGGAGGGCGGCGGAAGACGCCAAAAACATAAAG
4luc-NcoI-F	**CCCCCCATGG**CGAGGAGGAGGAGGGCGGCGGAAGACGCCAAAAACATAAAG
6luc-NcoI-F	**CCCCCCATGG**CGAGGAGGAGGAGGAGGAGGGCGGCGGAAGACGCCAAAAACATAAAG
8luc-NcoI-F	**CCCCCCATGG**CGAGGAGGAGGAGGAGGAGGAGGAGGGCGGCGGAAGACGCCAAAAACATAAAG
luc-NdeI-R	**CCCCCCATATG**TTTACACGGCGATCTTTCC
rfp - F	**GGAATTCCATATG**GCTTCCTCCGAAGACG
rfp - R	**CCGCTCGAG**TTAAGCACCGGTGGAGTGAC
6rfp-F	**GGAATTCCATATG**AGGAGGAGGAGGAGGAGGGCTTCCTCCGAAGACG
ampR-F	CCTGTCGCTTGCGGTATTCGGCGGCATCCGCTTACAGACA
ampR-R	CAGTGAGCGAGGAAGCGGAACGCTCAGTGGAACGAAAA
2blaluc-F	AATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGGAGGAGGGAAGACGCCAAAAAC
4blaluc-F	AATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGGAGGAGGAGGAGGGAAGACGCCAAAAAC
6blaluc-F	AATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGGAGGAGGAGGAGGAGGGAAGACGCCAAAAAC
8blaluc-F	AATGCTTCAATAATATTGAAAAAGGAAGAGTATGAGGAGGAGGAGGAGGAGGAGGAGGGAAGACGCCAAAAAC
LucAmp-R	ACGGGAGGGCTTACCATCTGGTTACACGGCGATCTTTCC
trc-F	CCACCATACCCACGCCGAAACAAACAAAAAGAGTTTGTAGAAACGC
trc-R	TTGCCGTTACGCACCACCCGGTATTTTCTCCTTACGCATCTGT
argW-F	TTGTGAGCGGATAACAATTTCACACGTCCTCTTAGTTAAATGGATATAACGAG
argW-R	GTGGCAGCAGCCTAGGTTAA*G*TCAACGATCTGAAGCGAACCAT
aspV - F	CCACCATACCCACGCCGAAACCTAAAAATAGCGACTTGGGC
aspV - R	TTGCCGTTACGCACCACCCTTGAACAGAGAAATGGTGGAA
T7-F	AGAATGAATCACCGATACGCAGATCTCGATCCCGCGAA
T7-R	CCTACGAGTTGCATGATAAAGACAAAAAACCCCTCAAGACCC
aspV-CCU-F	AGTCGGTTAGAATACCTGCCT***CCT***ACGCAGGGGGTCGC
aspV-CCU-R	GCGACCCCCTGCGT***AGG***AGGCAGGTATTCTAACCGACT
TAGluc-NcoI-F	**CCCCCCATGG**CGTAGAGGAGGGCGGCGGAAGACGCCAAAAACATAAAG
TDRS-F	**CATGCC ATGG**GCGTTCTGCCGCTTGACTCT
TDRS-R	**GGAATTCCATATG**TCAGTTATTCTCAGCCTTCTTCACA
FB-argW-F	**GGAATTCCATATG**GAAAAATTATAAAAACCCG
FB-argW-R	**CCGCTCGAG**GTCAACGATCTGAAGCG

Note:

underlined letters	the homologous sequence used for overlap extension PCR
blod letters	the restriction enzyme sites
italic and blod letters	the mutation points

Table S2. Vectors used in this study

Vector name	Description
pET-T7-2AGGluc	lucferase gene with 2 AGG codons inserted after the second codon was inserted into pET28a between *Nco*I and *Nde*I under the T7 promoter
pET-T7-4AGGluc	lucferase gene with 4 AGG codons inserted after the second codon was inserted into pET28a between *Nco*I and *Nde*I under the T7 promoter
pET-T7-6AGGluc	lucferase gene with 6 AGG codons inserted after the second codon was inserted into pET28a between *Nco*I and *Nde*I under the T7 promoter
pET-T7-8AGGluc	lucferase gene with 8 AGG codons inserted after the second codon was inserted into pET28a between *Nco*I and *Nde*I under the T7 promoter
pET-ampR	*amp*R operon from pUC18 was amplified and inserted into pET28a replacing a nonessential region
pET-bla-4AGGluc	the ORF of luciferase gene inserted 4 AGG codons after initial codon replacing the ORF of *amp*R gene on pET-bla
pET-bla-6AGGluc	the ORF of luciferase gene inserted 6 AGG codons after initial codon replacing the ORF of a*mp*R gene on pET-bla
pET-bla-8AGGluc	the ORF of luciferase gene inserted 8 AGG codons after initial codon replacing the ORF of *amp*R gene on pET-bla
pET-T7-RFP	wild type RFP gene was inserted into pET28a between *Nde*I and *Xho*I under the T7 promoter
pET-T7-6AGGRFP	RFP gene with 6 AGG codons inserted after initial codon was inserted into pET28a between *Nde*I and *Xho*I under the T7 promoter
pACYC-trc	*lac*I regulator, trc promoter and *rrn*B ternimator were amplified together from pTrc99b and inserted into pACYC184 replacing *Cm*R operon
pACYC-trc-argW	insert *arg*W after the trc promoter on pACYC-trc
pACYC-aspV	*asp*V operon was amplified from the genome of *E.coli* and inserted into pACYC184 replacing the *Cm*R operon
pACYC-T7-aspV	T7 promoter from pET28a was inserted into pACYC-aspV replacing a fragment of blank sequence
pACYC-T7-aspV(CCU)	mutated the anticodon part of *asp*V gene on pACYC-T7-aspV from GTC to CCT
pACYC-T7-4AGGluc-aspV(CCU)	luciferase gene with 4 AGG codons inserted after initial codon was inserted after T7 promoter on pACYC-T7-aspV(CCU)
pACYC-T7-6AGGRFP-aspV(CCU)	RFP gene with 6 AGG codons inserted after initial codon was inserted after T7 promoter on pACYC-T7-aspV(CCU)
pET-T7-TDRS	the truncated aspRS was amplified from the genome of *E.coli* and inserted after T7 promoter on pET28a using overlap extension PCR
pET-T7-2AGGluc-argW	*arg*W gene was amplified from *E.coli* genome and inserted after 2AGG-luc gene before T7 ternimator on pET-T7-2AGGluc
pET-T7-4AGGluc-argW	*arg*W gene was amplified from *E.coli* genome and inserted after 4AGG-luc gene before T7 ternimator on pET-T7-4AGGluc
pET-T7-6AGGluc-argW	*arg*W gene was amplified from *E.coli* genome and inserted after 6AGG-luc gene before T7 ternimator on pET-T7-6AGGluc

pET-T7-8AGGluc-argW	*arg*W gene was amplified from *E.coli* genome and inserted after 8AGG-luc gene before T7 ternimator on pET-T7-8AGGluc

Supplementary Figure legends

Fig. S1: Fatty acid metabolism pathway in *E. coli.* FabA, FabB, FabD, FabF, FabG, FabH, FabI, FabZ are the main enzymes of the fatty acid biosynthesis pathway in *E.coli*. FabB, FabF, Fab G, FabI, FabZ/FabA are mainly responsible for fatty acid elongation. TesA is the enzyme that is able to release free fatty acid by hydrolysis of acyl-ACP species. FabG, FabZ, FabI and TesA were picked as targets for manipulation in these experiments based on previous studies.

Fig. S2: RFP expression controlled by rare codon devices. (**A**) Constructs for testing rare codon devices. RFP containing 6 AGGs and $tRNA^{Arg}$ (CCU) were expressed under separate *T7* promoters (blue). (**B**) Cells containing *rfp* without the rare AGG codon and $tRNA^{Arg}$ (CCU) expressed RFP (upper row). RFP expression nearly ceased upon insertion of 6 AGG codons (S2A) (middle row). RFP expression was restored in cells containing pACYC-trc-argW (coded for $tRNA^{Arg}$ (CCU)) (bottom row). (**C-I**) Time-course assays of luciferase activity. Black line indicates luciferase activity in the cell carrying pET-T7-nAGGluc and pACYC-trc-argW (C-F, n = 2, 4, 6, 8) or pET-bla-*n*AGGluc and pACYC-trc-argW (G-I, n = 4, 6, 8). Red line indicates luciferase activity in the cell carrying pET-T7-nAGGluc (C-F, n = 2, 4, 6, 8) or pET-bla-*n*AGGluc (G-I, n = 4, 6, 8).

Fig S3: Protein levels of luciferase with various numbers of rare AGG codon insertions after the start codon. (A) SDS-PAGE of lysates of cells containing rare codon devices

shown in Fig. 1A. The upper bands (nAGGLuc) represent luciferase with various numbers of arginines at the N terminus. The lower bands are western blots of DnaK protein, which was chosen as the internal control. **(B)** The bands in Fig. S3A were quantified in terms of relative protein amounts using Quantity One® software.

Fig. S4: Control of luciferase expression via TDRS and mutated tRNA^{Asp} (GUC→CCU). (**A**) Sketch showing TDRS and mutated tRNA^{Asp} (GUC→CCU). (**B**) Luciferase activity was detected only in the cell carrying plasmids containing *luciferase* with 4 AGG codon insertions, mutated tRNA^{Asp} (GUC→CCU), and TDRS (right-hand column). Only the luciferase substrate (considered as background) was detected from the cell containing *luciferase* with 4 AGG codon insertions and mutated tRNA^{Asp} (GUC→CCU) (left-hand column).

Fig. S5: Control of kanamycin resistance gene expression using truncated MetRS and tRNA^{fMet} (CAT→TCG). (**A**) Sketch of the test system. The start codon of *KanR* was mutated to CGA to permit recognition by tRNA^{fMet} (CAT→TCG). The tRNA^{fMet} (CAT→TCG) was charged using truncated MetRS. (**B**) Expression of *KanR* (ATG→CGA) was controlled by truncated MetRS and tRNA^{fMet} (CAT→TCG). The left-hand figure shows a cell carrying truncated MetRS, tRNA^{fMet} (CAT→TCG), and *KanR* (ATG→CGA) grown on a plate containing kanamycin. The cell that carried only tRNA^{fMet} (CAT→TCG) and *KanR* (ATG→CGA) could not grow on the kanamycin plate showed in the right-hand figure. "N" and "M" indicate two independent clones.

Fig. S6: Component analysis of fatty acid products by GC-MS. Data for the strains **(A**) ZGIT, **(B)** 4Z4G4I4T, and **(C)** 4Z8G4I1T. The X-axis and Y-axis represent retention time and relative amounts, respectively.

Fig. S7: Analysis of the structures of aminoacyl-tRNA synthetases complexed with cognate tRNAs. Black arrows indicate the anticodon recognition domains of various aminoacyl-tRNA synthetases. Figures on the left depict natural aminoacyl-tRNA synthetases complexed with cognate tRNAs, while those on the right represent hypothesized truncated aminoacyl-tRNA synthetases without the anticodon recognition domain. (**A**) Arginyl-tRNA synthetase, (**B**) cysteinyl-tRNA synthetase, (**C**) glutaminyl-tRNA synthetase, (**D**) glutamyl-trna synthetase, (**E**) threonyl-tRNA synthetase, (**F**) tryptophanyl-tRNA, (**G**) tyrosyl-tRNA synthetase, and (**H**) methionyl-tRNA synthetase.

Fig. S1

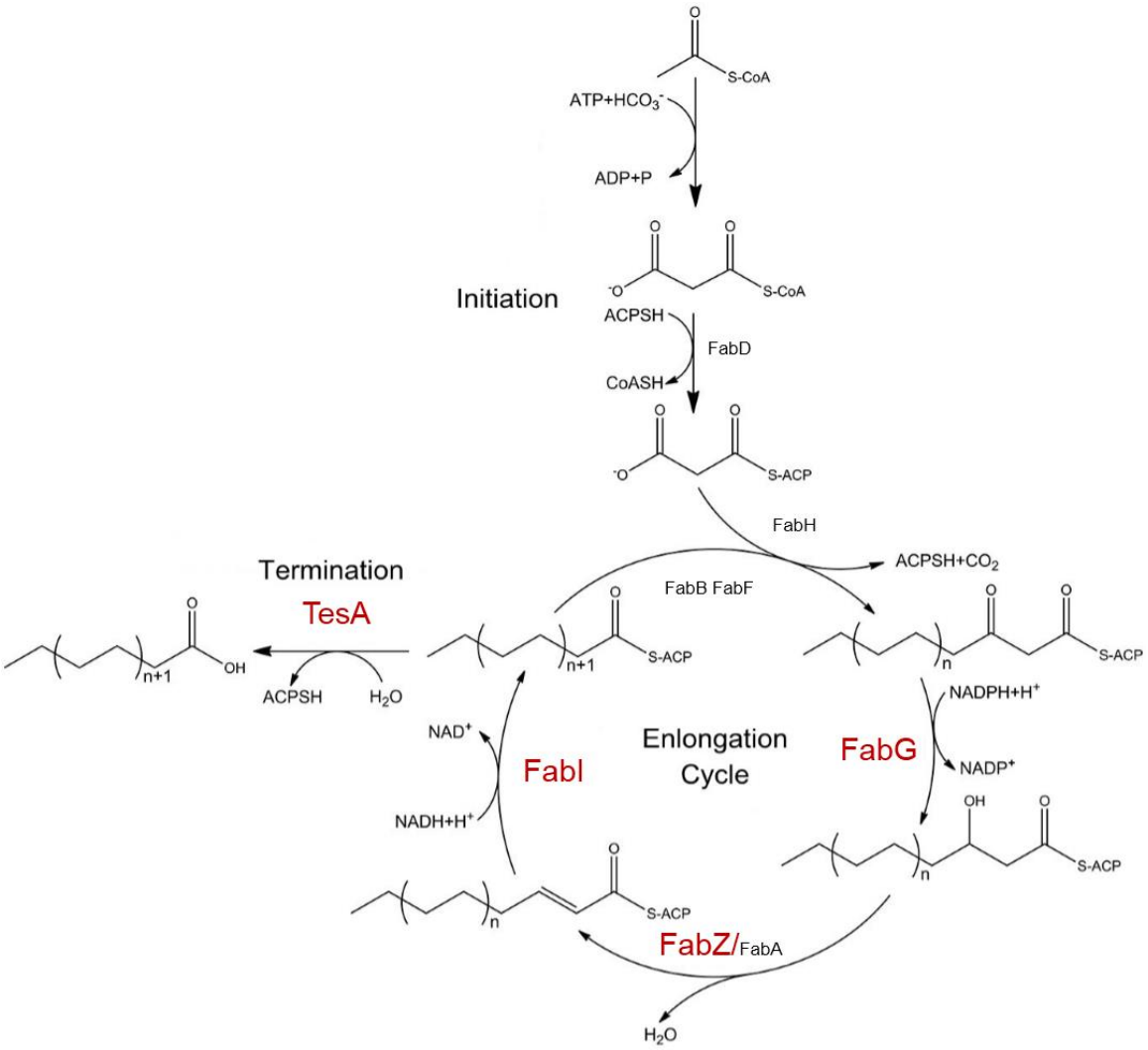

S-CoA
ATP+HCO3-
ADP+P
Initiation
ACPSH
FabD
CoASH
S-ACP
FabH
ACPSH+CO2
FabB FabF
Termination
TesA
n+1
OH
ACPSH
H2O
n
NADPH+H+
NADP+
FabG
Enlongation
Cycle
FabI
NAD+
NADH+H+
OH
FabZ/FabA
H2O

Fig. S2A

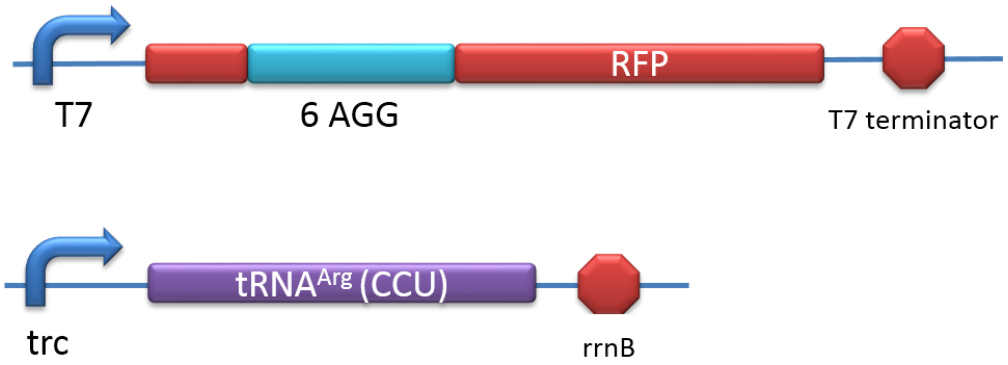


Fig. S2B

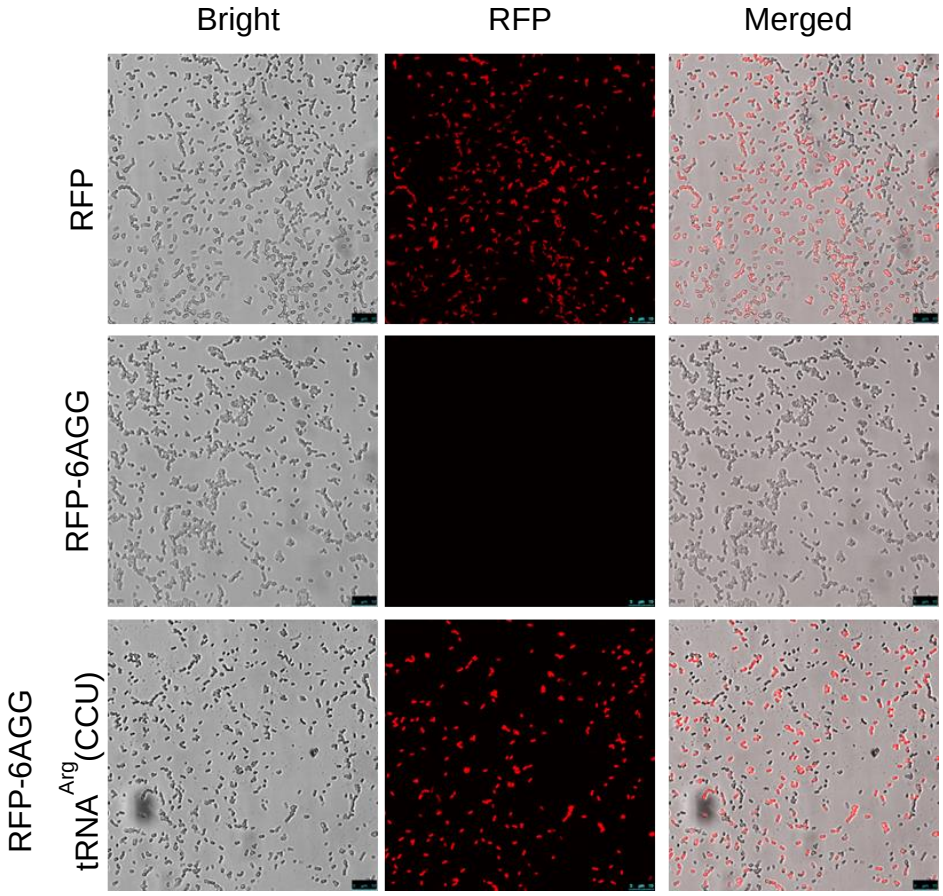


Fig. S2C

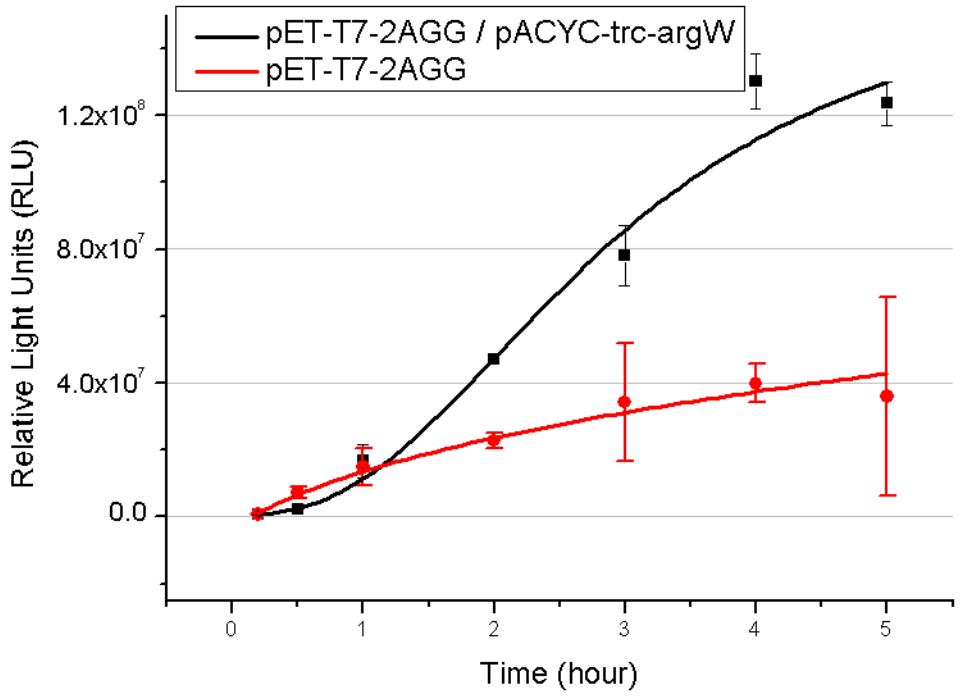


Fig. S2D

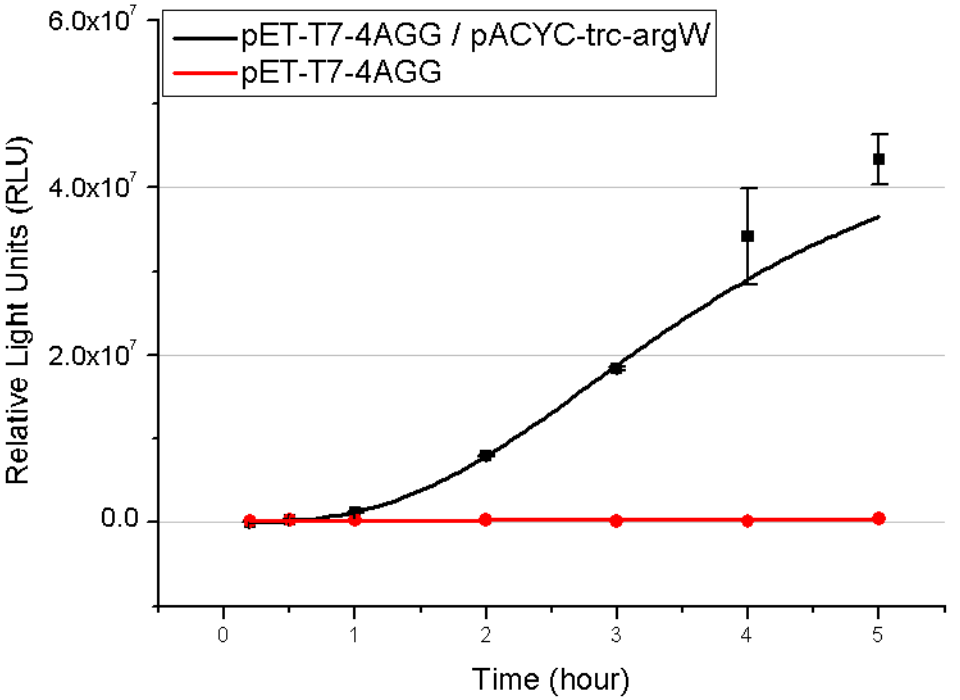


Fig. S2E

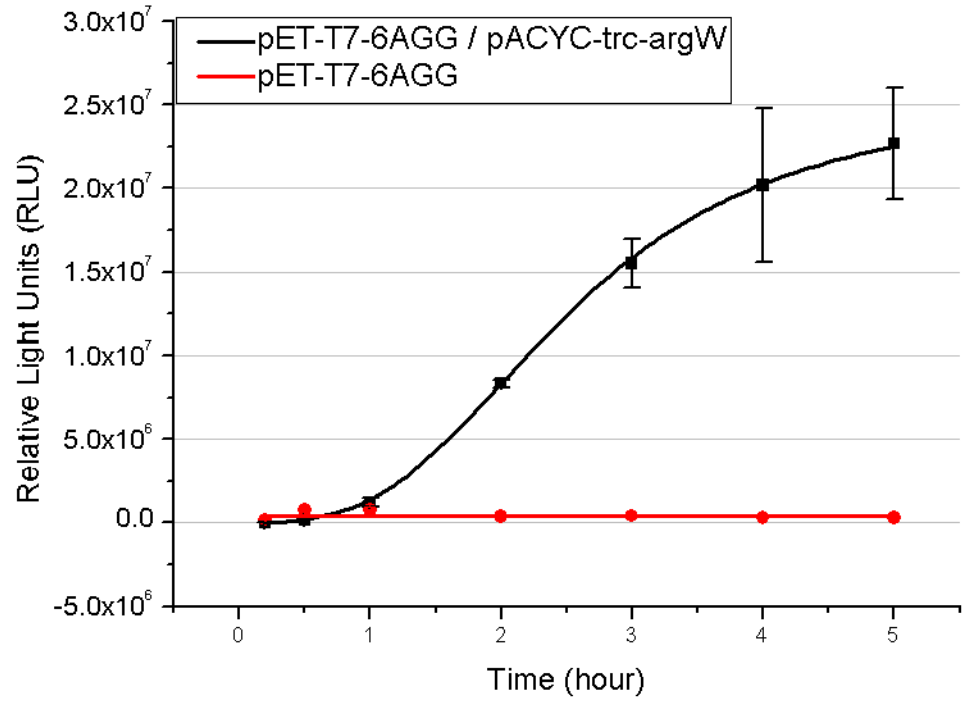


Fig. S2F

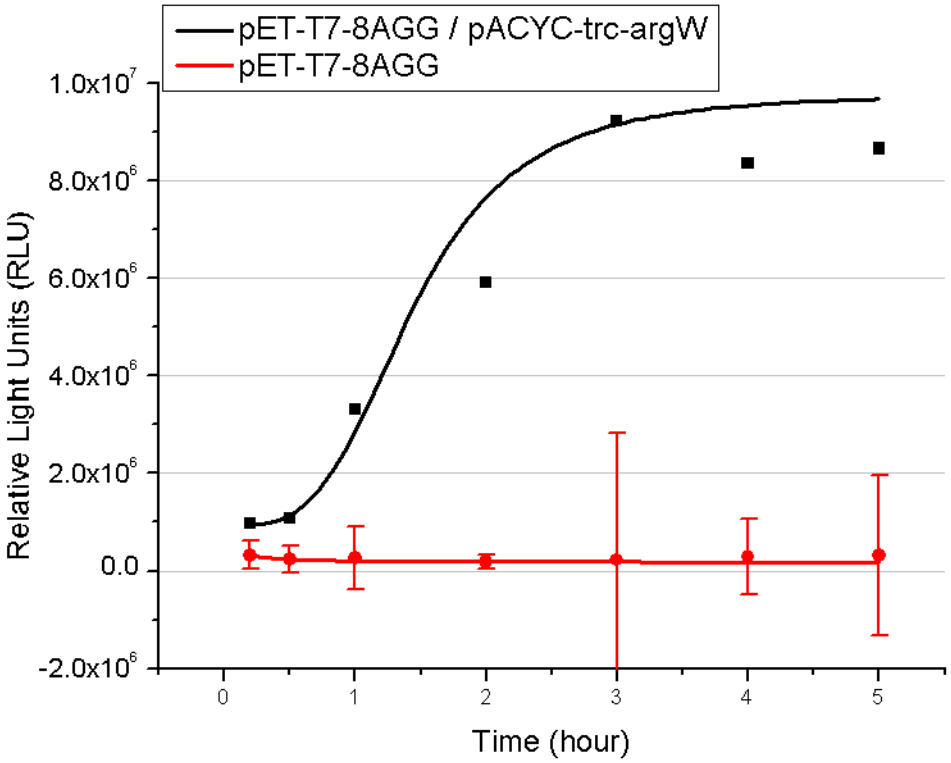


Fig. S2G

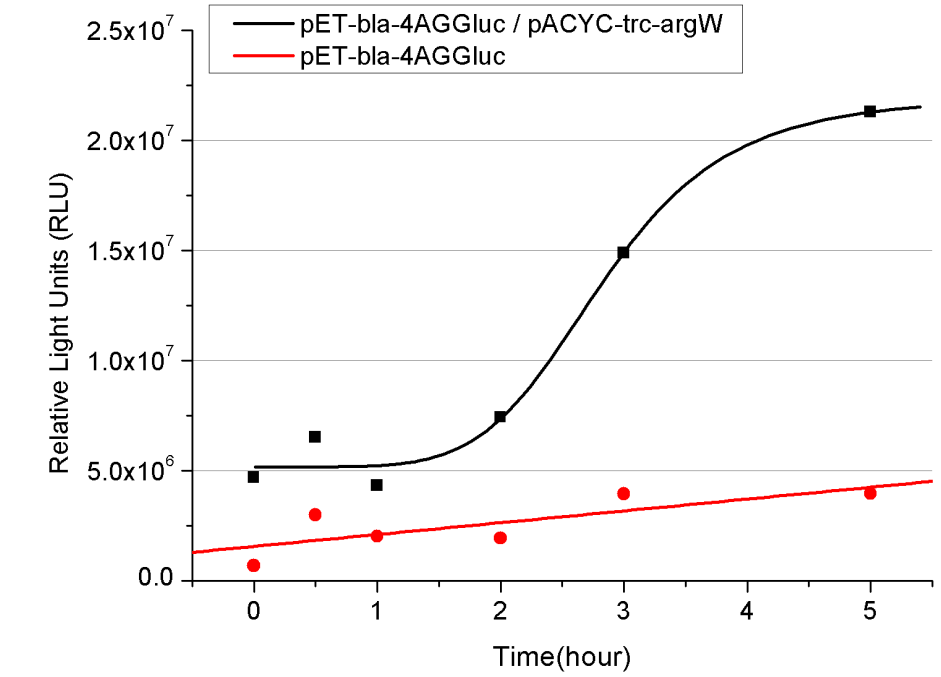


Fig. S2H

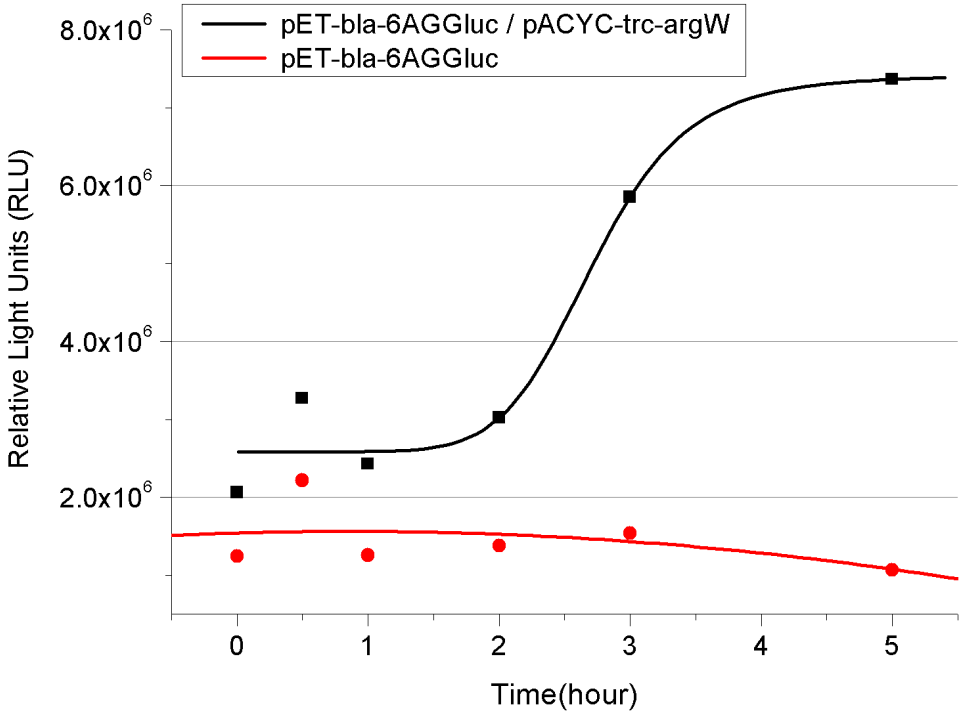


Fig. S2I

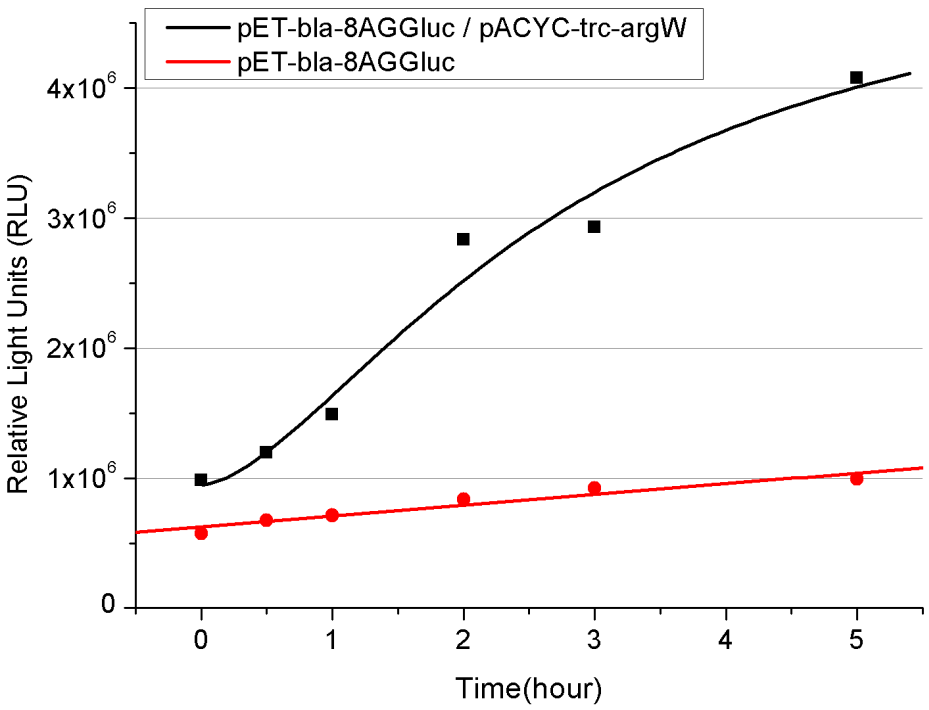

pET-bla-8AGGluc / pACYC-trc-argW
pET-bla-8AGGluc
Relative Light Units (RLU)
4x10^6
3x10^6
2x10^6
1x10^6
0
0
1
2
3
4
5
Time(hour)

Fig. S3A

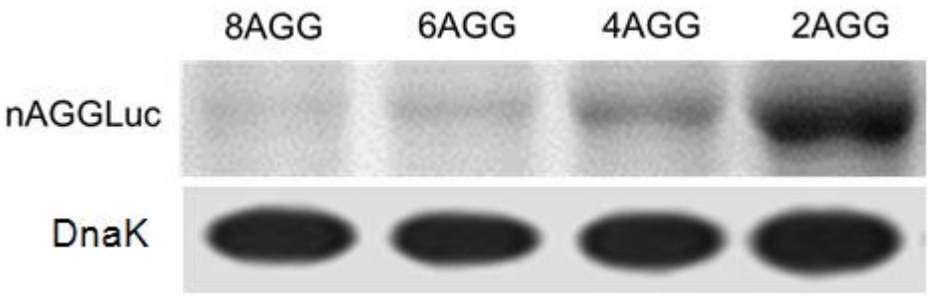


Fig. S3B

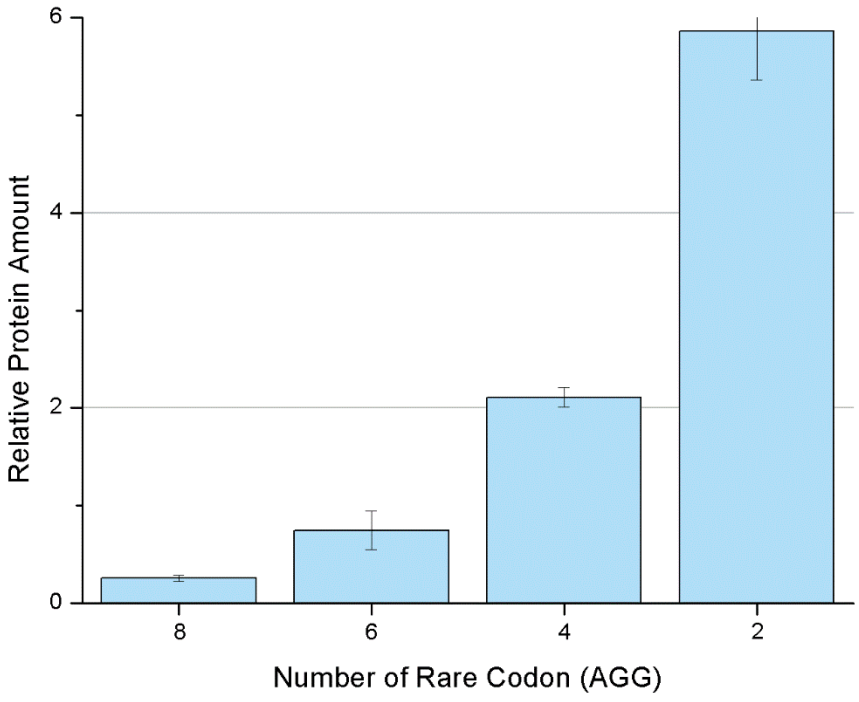


Fig. S4A

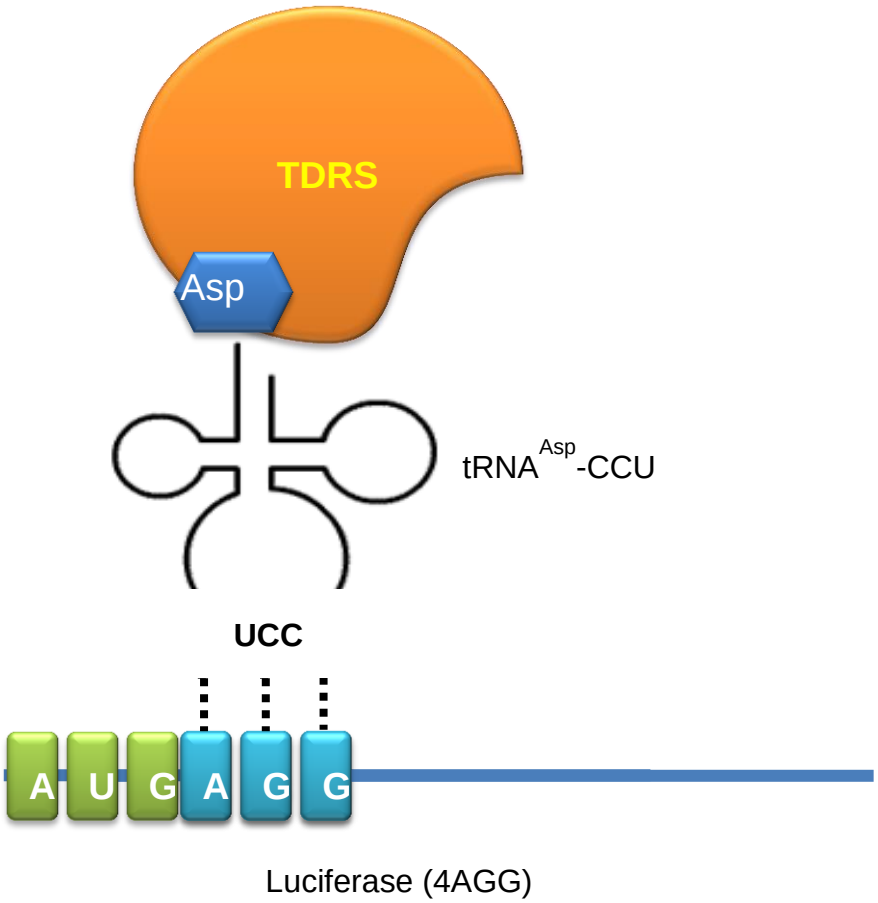


Fig. S4B

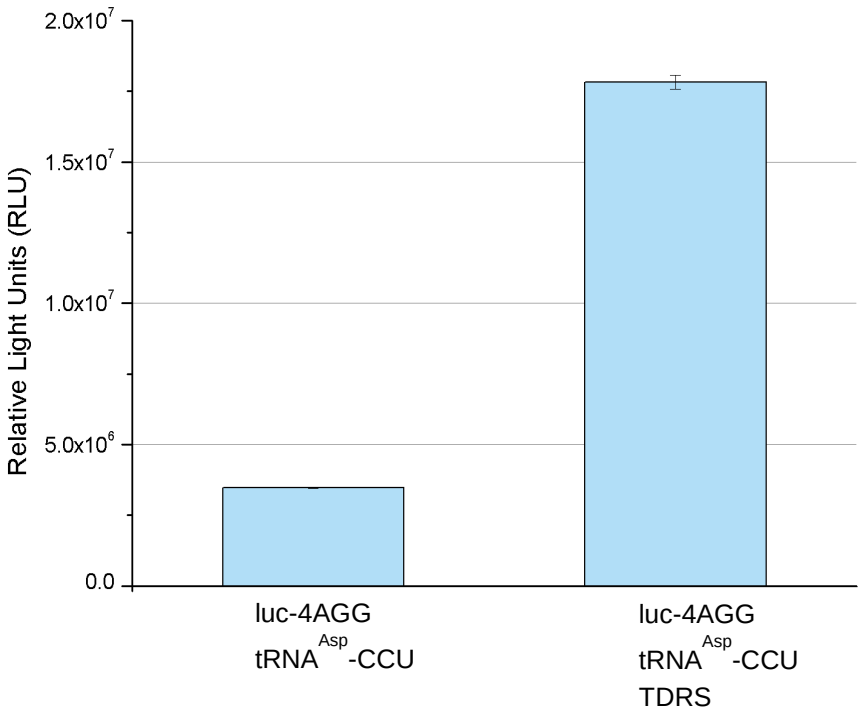


Fig. S5A

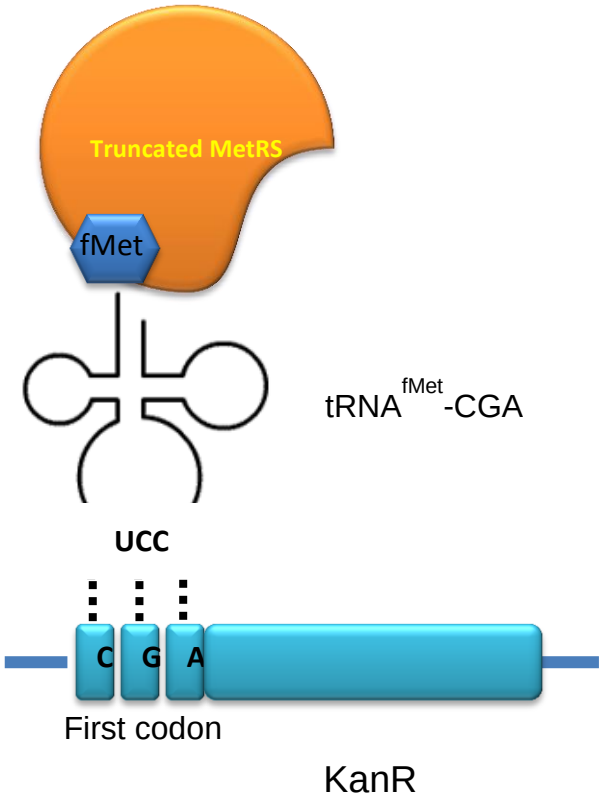
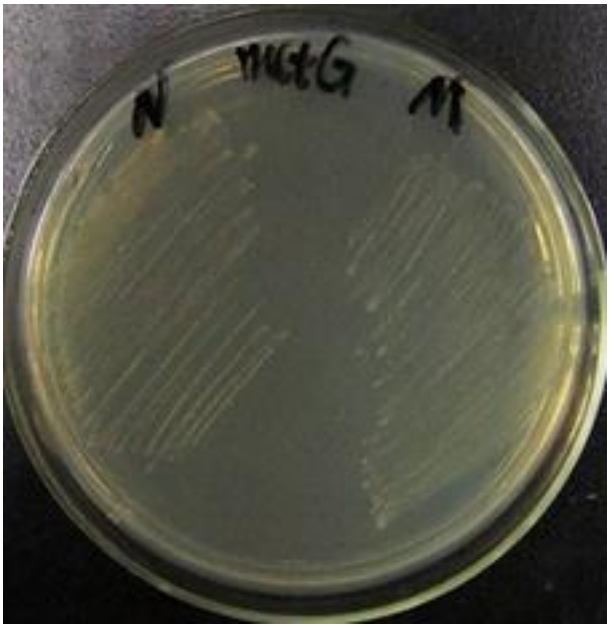


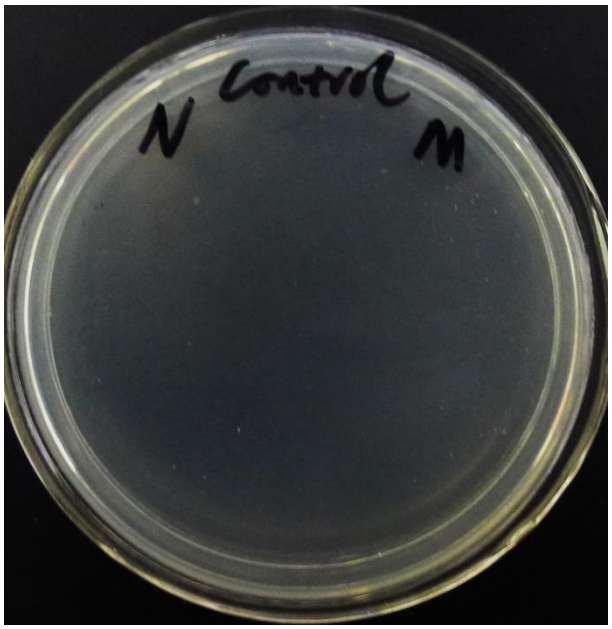
Fig. S5B



Trucated MetRS

tRNA fMet (CAT→TCG)

KanR (ATG→CGA)



tRNA fMet (CAT→TCG)

KanR (ATG→CGA)

Fig. S6A

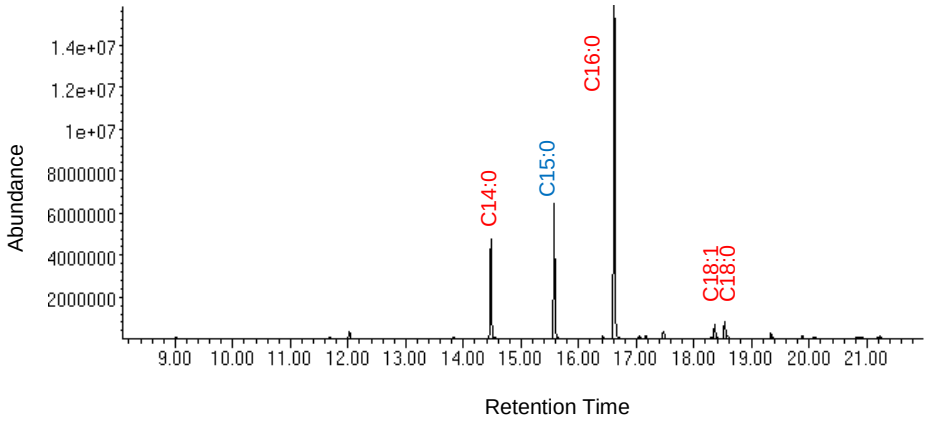


Fig. S6B

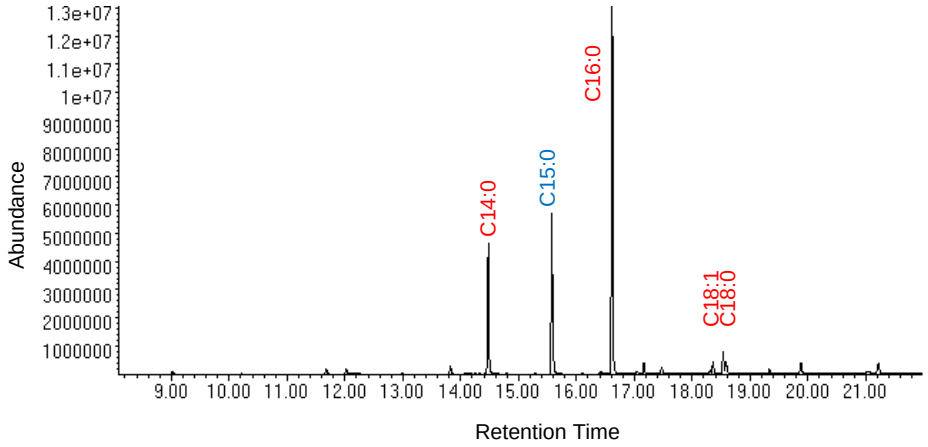


Fig. S6C

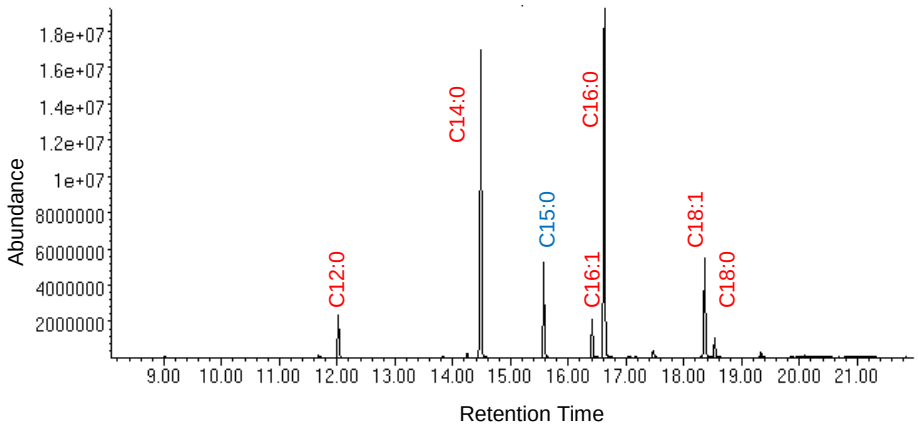
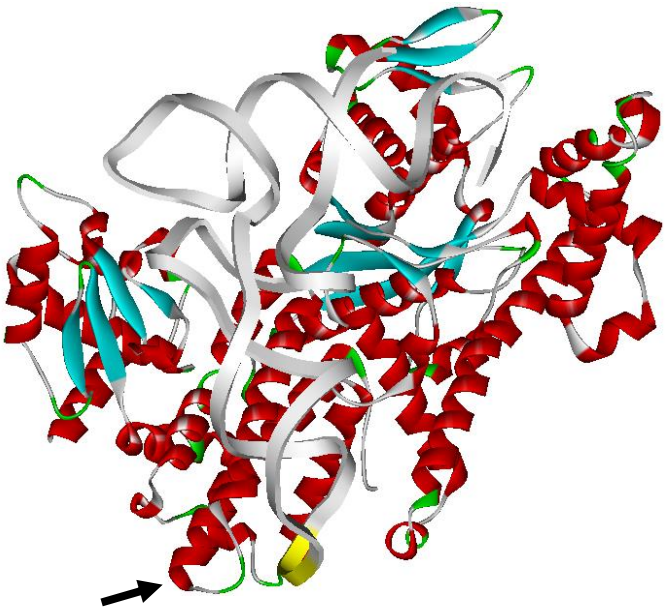
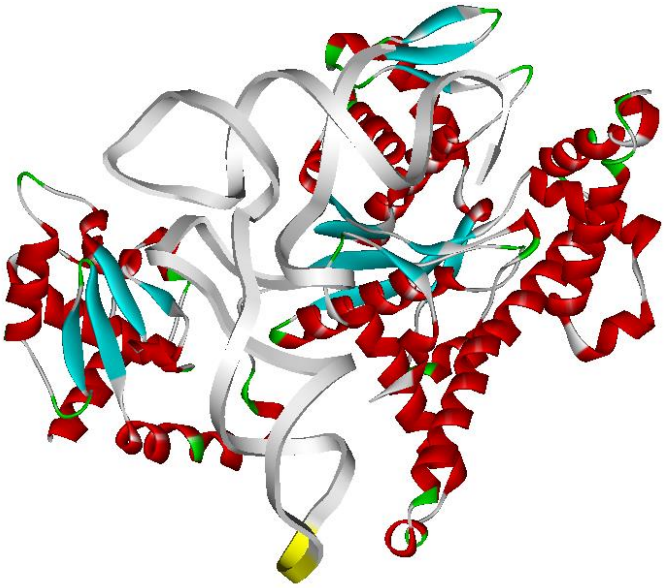


Fig. S7A

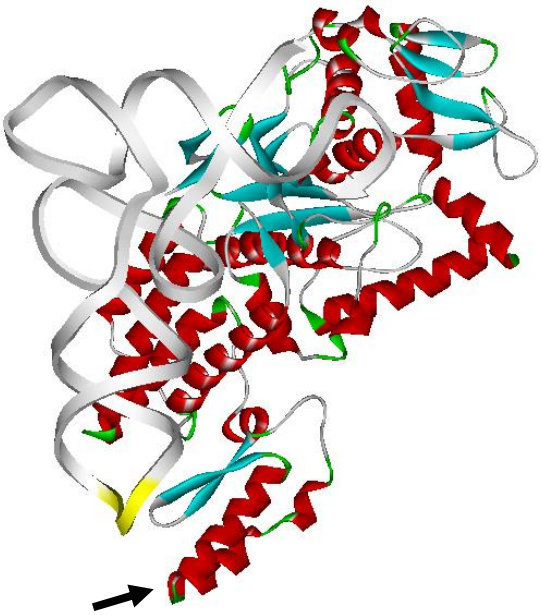


Arginyl-tRNA synthetase



Truncated Arginyl-tRNA synthetase
(anticodon recognition domain deletion (483-607))

Fig. S7B



Cysteinyl-tRNA synthetase

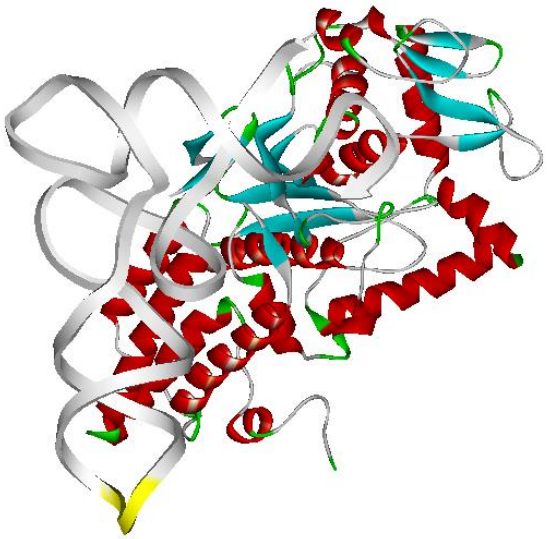


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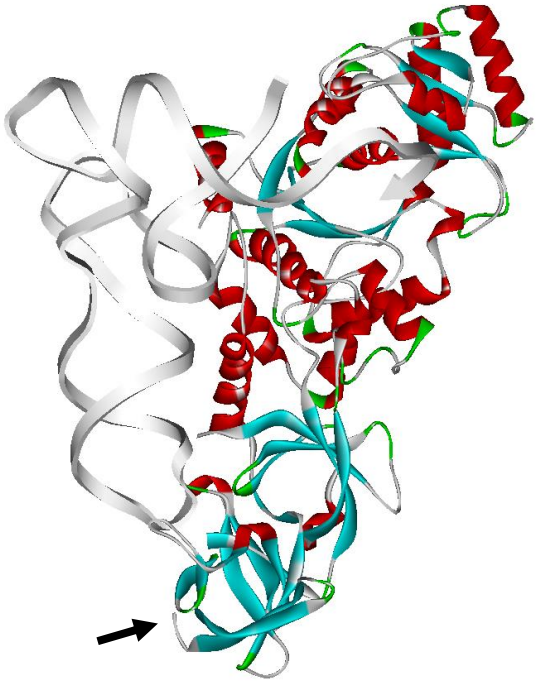
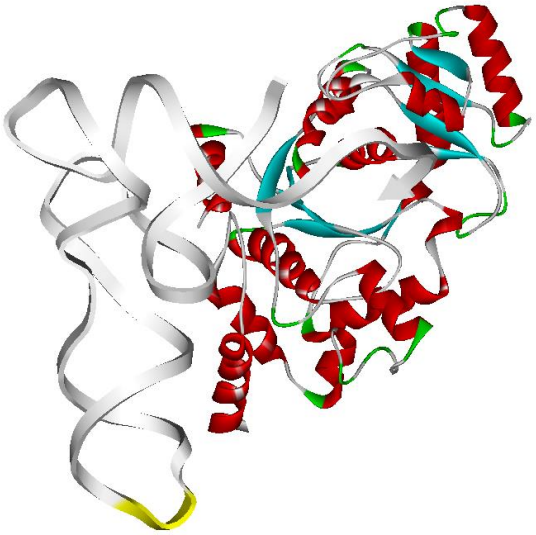
Fig. S7C



Glutaminyl-tRNA synthetase (PDB 1O0C)



Truncated Glutaminyl-tRNA synthetase
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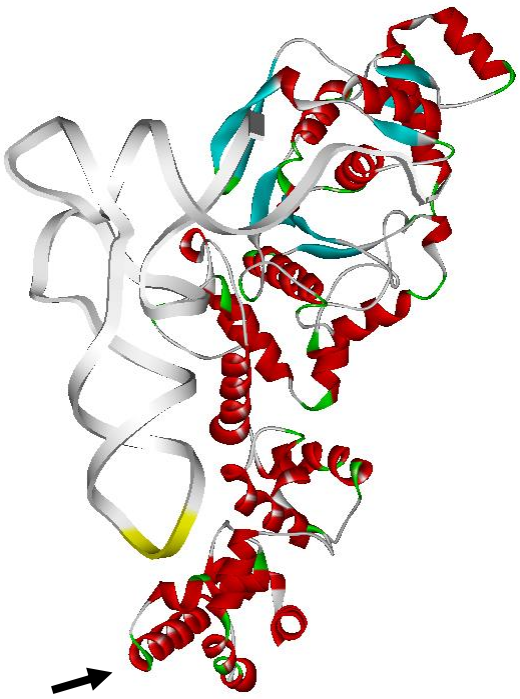
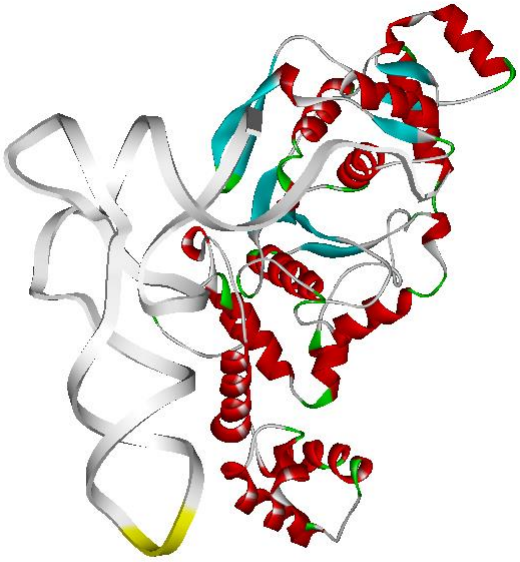
Fig. S7D



Glutamyl-trna synthetase (PDB 1G59)



Truncated Glutamyl-trna synthetase
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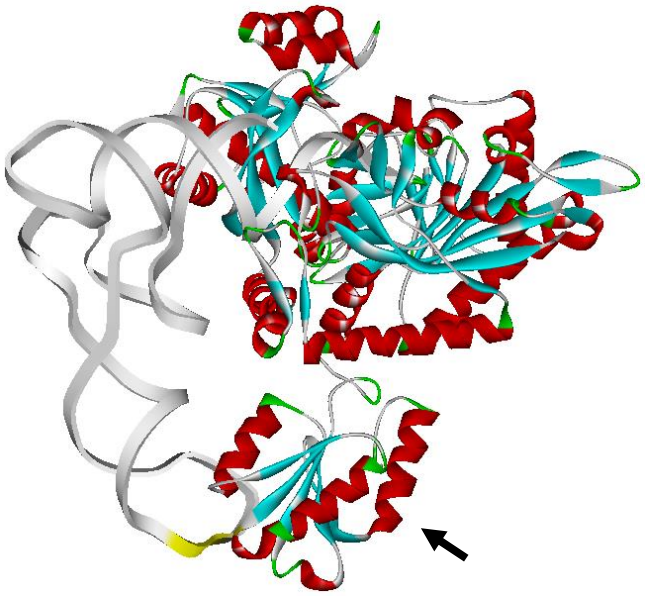
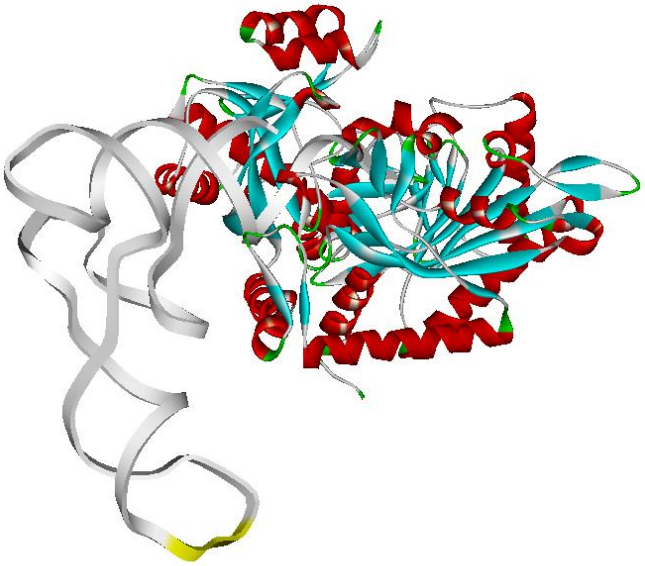
Fig. S7E

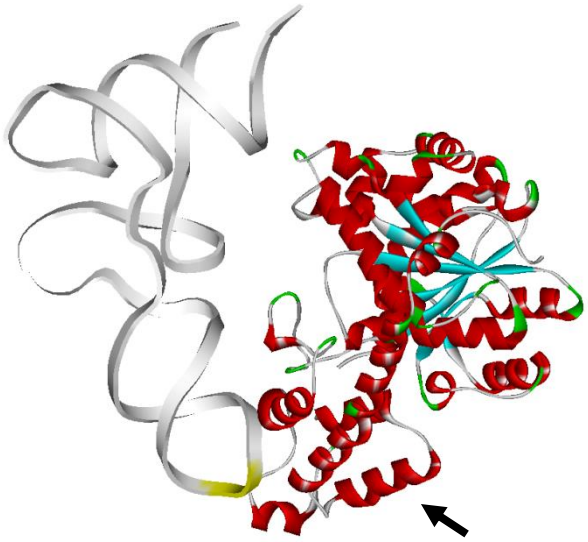


Threonyl-tRNA synthetase (PDB 1QF6)

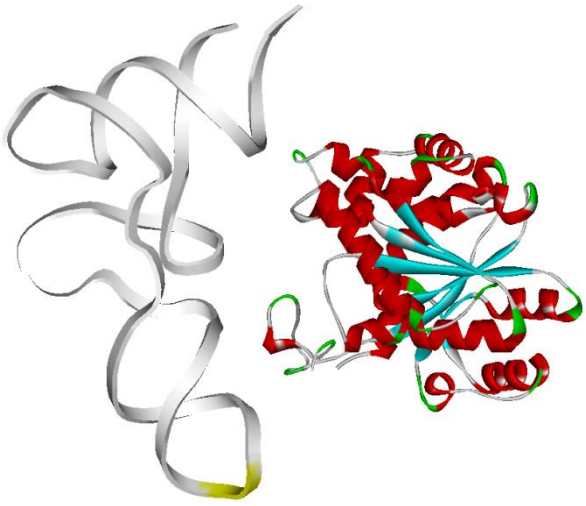


Truncated Threonyl-tRNA synthetase
(anticodon recognition domain deletion (536-642))

Fig. S7F

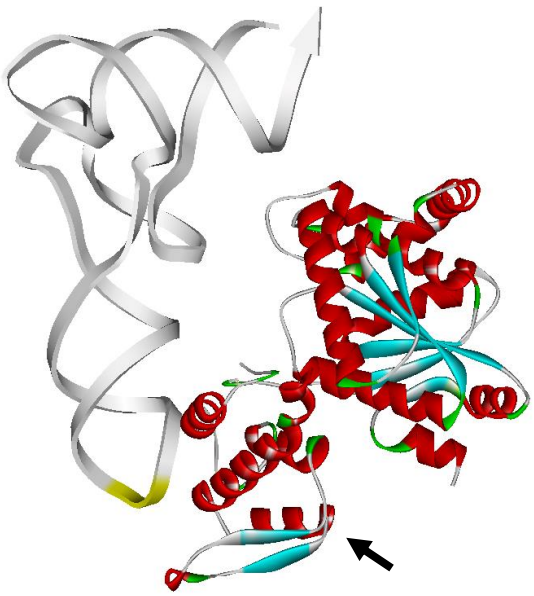


Tryptophanyl-tRNA synthetase (PDB 2AKE)

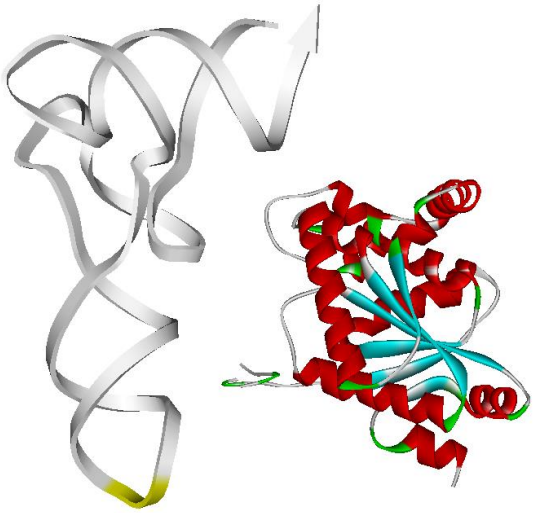


Truncated Tryptophanyl-tRNA synthetase
(anticodon recognition domain deletion (365-469))

Fig. S7G

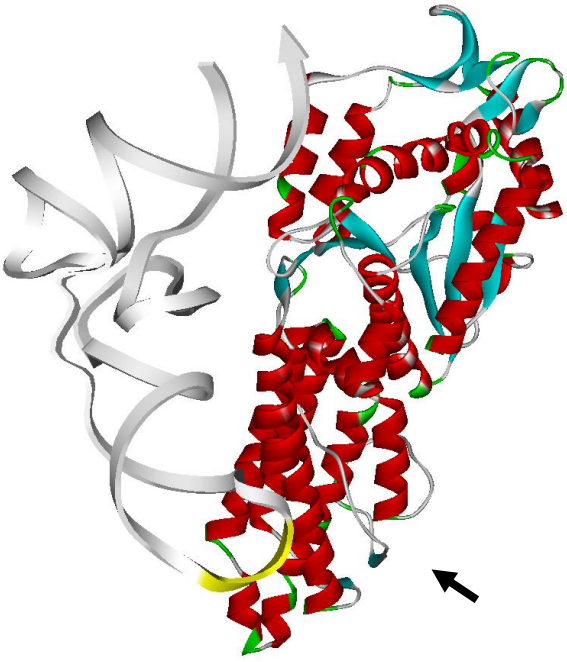


Tyrosyl-tRNA synthetase (PDB 1J1U)

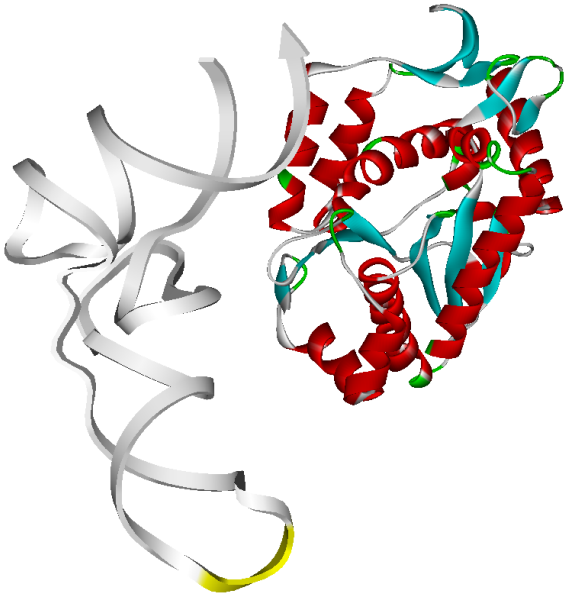


Truncated Tyrosyl-tRNA synthetase
(anticodon recognition domain deletion (219-306))

Fig. S7H



Methionyl-tRNA synthetase (PDB 2CSX)



Truncated Methionyl-tRNA synthetase
(anticodon recognition domain deletion (336-497))